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Diabetes mellitus-associated changes in ovarian ERα expression during puberty and adulthood

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The objective: **to study changes in estrogen receptor alpha (ERα) expression in the ovaries of rats of different ages under conditions of diabetes mellitus (DM) and comorbidity and to determine their association with ovarian reproductive dysfunction.**
Materials and methods**. The study was conducted on 40 female white laboratory rats aged 2 and 6 months, which were divided into 4 groups: Group 1 – rats with streptozotocin-induced DM (SIDM) combined with immobilization stress (IS), Group 2 – rats with SIDM, Group 3 – rats with IS, and Group 4 – control animals.**
Results**. In the control group, ERα expression in 2-month-old rats was significantly lower compared to 6-month-old animals. Weakly positive ERα cells were detected in the interstitium and in the theca cells of secondary follicles, whereas in 6-month-old rats, theca cells of all follicle types demonstrated moderate and strong positive ERα expression.**
Under SIDM conditions, a decrease and abnormal expression of ERα were observed in animals of different ages. In sexually mature individuals, this was manifested by a reduction in the pool of primordial follicles, Graafian follicles, and corpora lutea, along with an increased number of atretic follicles and the appearance of cystic follicles, indicating depletion of the ovarian reserve and a possible decline in fertility in this group. In 2-month-old rats, a decrease in the number of secondary and Graafian follicles and the appearance of cystic follicles were observed, which may indicate delayed sexual maturation. A single exposure to stress led to a decrease in ERα expression, which was particularly pronounced in peripubertal rats. In contrast, the combination of SIDM and IS resulted in increased aberrant ERα expression, accompanied by pronounced changes in folliculogenesis in both age groups.
Conclusions**. SIDM leads to decreased and aberrant ERα expression and impaired folliculogenesis. Isolated stress reduces ERα expression without significant morphological changes, whereas the combination of SIDM and IS leads to enhanced ovarian reproductive dysfunction.**
Keywords: *estrogen receptors, diabetes mellitus, stress, polycystic ovary syndrome, female reproductive system, ovaries, gynecological pathology.*

Зміни експресії ERα в яєчниках, асоційовані з цукровим діабетом, у пубертатному періоді та дорослому віці

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Мета дослідження: вивчення змін експресії рецептора естрогену альфа (estrogen receptor alpha – ERα) в яєчниках щурів різного віку за умов цукрового діабету (ЦД) й коморбідності та з'ясування зв'язку цих змін із репродуктивними дисфункціями яєчників.
Матеріали та методи. Дослідження проведено на 40 самках білих лабораторних щурів віком 2 і 6 міс., яких було розподілено на 4 групи: 1-ша група – щури зі стрептозотоциновим ЦД (СЦД) та іммобілізаційним стресом (ІС), 2-га – зі СЦД, 3-тя – з ІС, 4-та – контрольні тварини.
Результати. У контрольній групі 2-місячних щурів експресія ERα була значно нижчою порівняно з 6-місячними тваринами. ERα-слабкопозитивні клітини виявлялися в інтерстиції та теці вторинних фолікулів, тоді як у 6-місячних щурів текальні клітини всіх фолікулів демонстрували інтенсивну або помірну позитивну експресію ERα.
При СЦД у тварин різного віку спостерігалися зниження та аномальна експресія ERα. У статевозрілих особин це проявлялося зменшенням пулу примордіальних фолікулів, Граафових пухирців та жовтих тіл, збільшенням кількості атретичних фолікулів і появою кістозних фолікулів, що свідчить про виснаження овариального резерву та можливе зниження фертильності у цій групі. У 2-місячних щурів відзначали зменшення кількості вторинних фолікулів і Граафових пухирців, а також появу кістозних фолікулів, що може вказувати на затримку статевого дозрівання. Одноразовий вплив стресу призводив до зниження експресії ERα, що було найбільш вираженим у щурів перипубертатного віку. Натомість поєднання СЦД та ІС зумовлювало підвищену аномальну експресію ERα, що супроводжувалося вираженими змінами фолікулогенезу в обох вікових групах.
Висновки. СЦД спричиняє зниження та аномальну експресію ERα, а також порушення фолікулогенезу. Стрес ізольовано знижує експресію рецептора ERα без суттєвих морфологічних змін, тоді як поєднання СЦД та ІС призводить до вираженої репродуктивної дисфункції яєчників.
Ключові слова: *естрогенові рецептори, цукровий діабет, стрес, синдром полікістозних яєчників, репродуктивна система жінки, яєчники, гінекологічна патологія.*

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Both type 1 and type 2 diabetes mellitus (DM) represent a global medical and social problem, as they are associated with an increased risk of cardiovascular complications, disability, mortality, and a financial burden on the healthcare systems of many countries [1–4]. Furthermore, DM is associated with menstrual irregularities in women of reproductive age, including polycystic ovary syndrome (PCOS) [5, 6], menstrual cycle disorders [7–9], infertility [1, 8] and early menopause [10, 11].

DM is one of the most common chronic endocrine conditions in children and adolescents [12, 13]. According to the International Diabetes Federation, nearly 1.8 million individuals under the age of 20 are living with type 1 DM, with approximately 150,000 new cases being recorded annually [12]. In adolescents with type 1 diabetes, menstrual cycle disorders are observed significantly more frequently than in healthy girls [10, 14]. This usually manifests as a delay in the onset of menarche, the development of oligomenorrhea, and a prolongation of the menstrual cycle [7, 11]. Furthermore, it has been established that poor metabolic control, manifested by a 1% increase in HbA1c levels, is associated with a significant increase in the likelihood of oligomenorrhea and an increase in the average duration of the menstrual cycle by approximately 5.1 days [14, 15].

Despite significant progress in the treatment of DM, excessive insulin administration can lead to impaired reproductive function, as insulin acts as a gonadotropin on the ovarian theca cells, resulting in the development of hyperandrogenism and suppression of normal ovulation [16, 17].

It has been demonstrated that glucose homeostasis is modulated by estrogen receptors alpha and beta (ERα and ERβ) via the specificity protein 1 (Sp1), which encodes glucose transporter type 4 (GLUT4) [18, 19]. Researchers suggest that ERα increases GLUT4 expression via Sp1, while ERβ has the opposite effect [20, 21]. However, the role of ER in changes to ovarian function in DM during puberty remains unclear.

The objective: to elucidate whether there are changes in ERα expression in the ovaries of rats of different ages with DM in the context of comorbidity, and whether these changes are associated with ovarian reproductive dysfunction.

MATERIALS AND METHODS

A total of 40 female albino laboratory rats, aged 2 and 6 months, were included in the experiment and evenly allocated into 4 groups (n = 5 per group). Group 1 consisted of animals with combined pathology, namely streptozotocin-induced DM (SIDM) together with immobilization stress (IS). Group 2 included rats with SIDM only, while Group 3 comprised animals exposed solely to IS. Group 4 served as the control and included healthy rats.

In Groups 1 and 2, DM was induced via a single intraperitoneal administration of streptozotocin "SIGMA" (USA). The dosage was 60 mg/kg for 6-month-old rats, prepared in 0.1 M citrate buffer, and 70 mg/kg for 2-month-old animals. In Groups 1 and 3, IS was induced by placing the rats in a confined plastic container for 5 hours daily [22]. In Group 1, DM was first established, and IS was introduced starting from day 14 of the study.

The experiment was conducted in the vivarium of the Ivano-Frankivsk National Medical University (IFNMU). Samples of ovarian tissue and blood were obtained on day 14 from the start of the experiment in Groups 1, 2, and 4. For Group 3, sampling was carried out during the initial days following the onset of IS. All collections were conducted in the early morning hours (7:00–8:00 a.m.) prior to feeding. Glucose levels were measured daily in the fasting state using a drop of blood obtained from the tail vein and analyzed with a portable Accu-Chek glucometer (Roche Diabetes Care, Germany). In addition, at the end of the experiment, glucose concentration was determined using the glucose oxidase method at the Center of Bioelementology of IFNMU. The levels of glycated hemoglobin (HbA1c) and cortisol were determined in the clinical diagnostics laboratory "Medlux". The concentration of HbA1c in the blood was determined using the "ACCENT-200 HbA1c DIRECT" diagnostic kit (PZ Cormay S.A., Poland). Serum cortisol levels were determined by enzyme-linked immunosorbent assay (ELISA) using the "EIA-1887, Cortisol ELISA" kit (DRG International, Germany).

Rat ovarian tissue samples were fixed in a 10% formalin solution (pH 7.4) for 24 hours. The tissues were then embedded in paraffin blocks using a standard technique with a HistoStar Embedding Station (Thermo Fisher Scientific, USA). Sections from each sample were prepared for general histological staining with hematoxylin and eosin (H & E) as well as for subsequent immunohistochemical analysis.

All animal experiments will be conducted comply with the requirements of the ethics committee of the IFNMU (Protocol No. 128/22 dated September 22, 2022), following the guidelines of the EU Directive 2010/63/EU for animal experiments, the European Convention for the Protection of Vertebrate Animals Used for Research and Other Scientific Purposes (Strasbourg, 1986). The stage of the estrous cycle was identified using vaginal cytology [22].

We used histological methods (H & E). To evaluate ovarian architecture and follicle number, sections stained with H & E were examined. Ten fields from three randomly selected sections of each ovary were examined at original magnifications of × 100 and × 200 to determine the number of follicles at different stages of maturation [22]. For the detection of ERα, antibodies against ERα (clone E115, 1:200, Abcam, United Kingdom) were used according to the manufacturer's instructions, allowing semi-quantitative assessment in tissue sections. ERα expression intensity in the ovary was evaluated using the histological score (HSCORE) method: 0 – no staining; 1 (+/–) – very weak staining (ERα expression may be heterogeneous, with both positive and negative cells present within the same tissue section); 2 (+) – weak staining; 3 (++) – moderate staining; 4 (+++) – intense staining [22].

All sections were evaluated with an optical microscope (Leica DM750) and monitored with attached digital camera (ToupCam 5.2M UHCCD C-Mount Sony sensor, ToupTek Photonics, China). Statistical analysis was performed using the statistical package Statistica 12 (StatSoft Inc., Tulsa, OK, USA). To assess differences between groups, the Mann–Whitney U test was used. The sample parameters presented in the text are denoted as M ± SD, where M represents the sample mean, SD the standard deviation, and p the achieved level of statistical significance.

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RESULTS AND DISCUSSION

The results of the biochemical analyses demonstrated the development of DM in rats of Groups 1 and 2 (Table 1). In animals from Group 3, elevated cortisol levels reflected a stress response induced by immobilization (Table 1). It should be noted that in 2-month-old rats, cortisol concentrations in Groups 1–3 were higher compared to 6-month-old animals (Table 1), which may indicate age-related differences in the stress response within this age category. Furthermore, rats with comorbid pathology exhibited the highest levels of glucose, HbA1c, and cortisol in comparison with all other experimental groups.

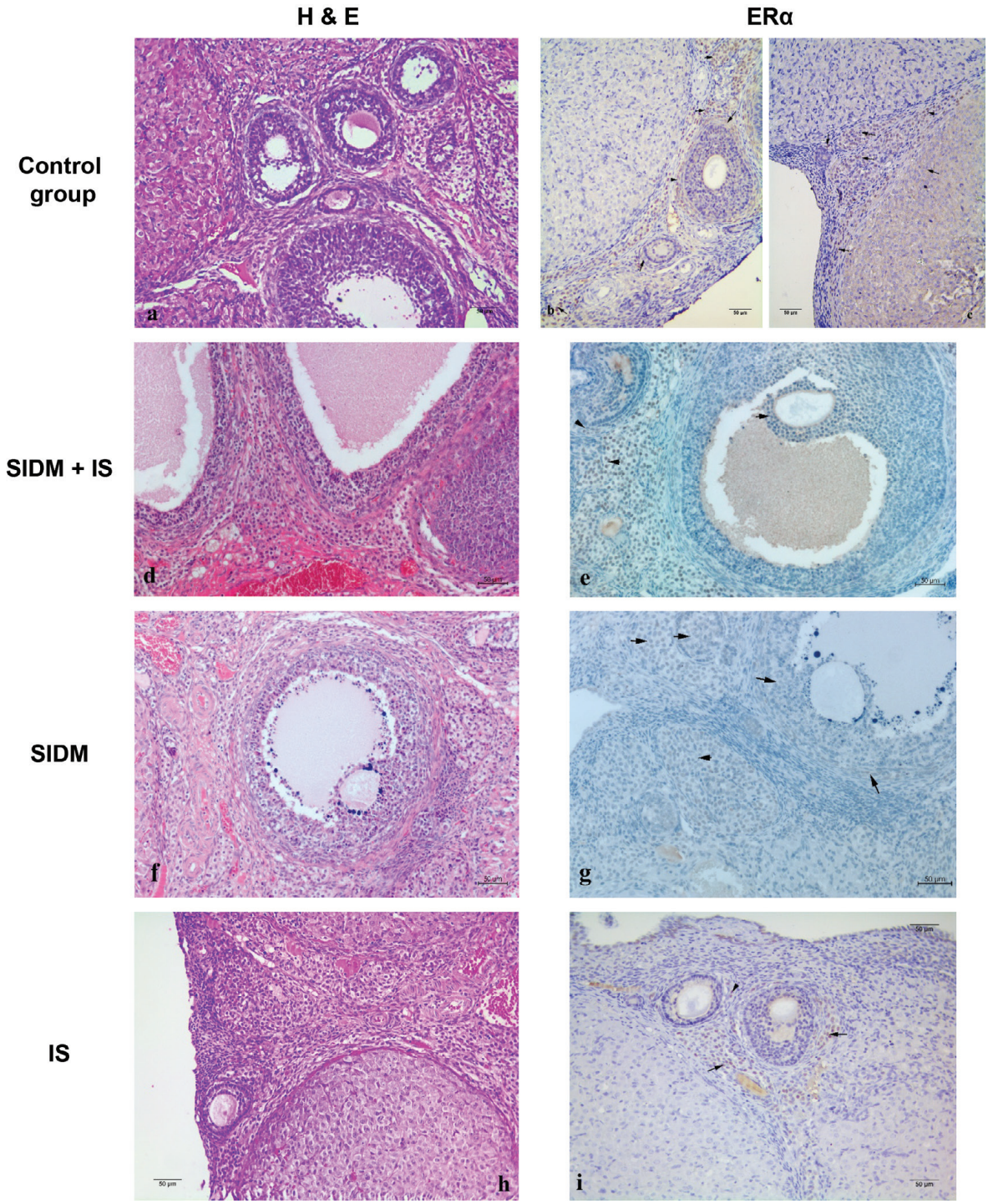

Fig. 1. Features of ovarian histoarchitecture in the control group (a) and abnormalities in folliculogenesis in the experimental groups (d, f, h) are shown. The pattern of ERα expression (arrow) in the control group (b, c) and its altered expression in the ovarian cortex of experimental groups of 6-month-old rats (e, g, i) are also presented

Notes: H & E – hematoxylin and eosin; ERα – estrogen receptor alpha; SIDM – streptozotocin-induced diabetes mellitus; IS – immobilization stress.

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As early as the 14th day of SIDM development, a quantitative restructuring of the ovarian cortical cytoarchitectonics is observed in Groups 1 and 2 of 6-month-old rats. Cystic follicles appear (Fig. 1d), and the number of primordial and primary follicles decreases compared with control values (Fig. 2). At the same time, there is no significant difference in the number of secondary and atretic follicles. In secondary and tertiary follicles, edema and detachment of granulosa cells into the follicular antrum can be observed (Fig. 1f, g). Such changes are evidently linked not only to metabolic changes in the animals' bodies but also to impaired microcirculation in the ovaries. Of particular note is the marked hyperemia of both the ovarian parenchyma and the cortex, resulting from erythrocyte sludge in the microvessels (Fig. 1d, f). In rats of Group 3, which were subjected to a single stressor, no significant morphometric changes in the ovarian cortex were observed (Fig. 2).

In the control group of animals, ERα expression is most pronounced in the interstitial cells and theca interna cells of all follicles (Fig. 1b, c). In the corpus luteum, ERα expression clearly depends on its stage of development and ranges from weak to moderate (Table 2). No marker was detected in oocytes or granulosa cells. In rats with comorbid pathology (Group 1), ERα expression was more pronounced in primary and atretic follicles compared to the control group. Furthermore, weak ERα expression was detected in the granulosa cells of the cumulus oophorus of Graafian follicles (Fig. 1e). In contrast, in rats with SIDM and IS, ERα expression in ovarian tissue was reduced (Fig. 1g, i). Furthermore, abnormal ERα expression was observed in individual granulosa cells of primary and secondary follicles in stressed rats.

An examination of the histological structure of the ovaries in peripubertal rats reveals a marked difference in the morphology of folliculogenesis (Fig. 2). This is primarily

Table 1

Biochemical parameters in experimental rats

Age, months	Group	Glucose, mmol/L	HbA1c, %	Cortisol, ng/mL
6	1	15.61 ± 2.23*	7.21 ± 0.72*	30.07 ± 2.93*
	2	13.53 ± 2.13*#	6.12 ± 0.48*#	18.21 ± 2.09*#
	3	5.45 ± 0.73#	2.18 ± 0.32#	28.49 ± 2.34*
	4	4.35 ± 0.52	2.03 ± 0.17	10.08 ± 1.13
2	1	15.37 ± 1.48*	6.78 ± 0.29*	47.61 ± 2.17*
	2	14.26 ± 1.12*	6.57 ± 0.53*	41.13 ± 3.17*#
	3	4.07 ± 0.26#	2.79 ± 0.15#	30.56 ± 20.25*#
	4	3.78 ± 0.23	2.34 ± 0.15	9.37 ± 1.08

Notes: * – significant in compare with Group 4 ($p < 0.05$); # – significant in compare with Group 1 ($p < 0.05$).

Table 2

Immunohistochemical evaluation of ERα in the ovary of mature rats

Follicular development	Cell type	Research groups			
		Control	SIDM + IS	SIDM	IS
Primordial	Oocyte	–	–	–	–
	Follicular cells	+	+	+	–
Primary	Oocyte	–	–	–	–
	Granulosa cells	–	–	–	+/–
	Theca cells	+	++	+/–	+
Secondary	Oocyte	–	–	–	–
	Granulosa cells	–	–	–	+/–
	Theca interna	++	+	+	++
	Theca externa	+	++	+	–
Graafian	Oocyte	–	–	–	–
	Granulosa cells	–	+/–	–	–
	Theca interna	++	++	+	+
	Theca externa	+	+	+	–
Atretic	Oocyte	–	–	–	–
	Granulosa cells	+	+	+	–
	Fibrous theca	+	++	+	+
Corpus luteum	Luteal cells	+	+	–	–
Interstitium		+++	+++	++	+

Notes: ERα expression levels in individual structures are presented as follows: 0 – no staining; 1 (+/–) – very weak staining; 2 (+) – weak staining; 3 (++) – moderate staining; 4 (+++) – intense staining; SIDM – streptozotocin-induced diabetes mellitus; IS – immobilization stress.

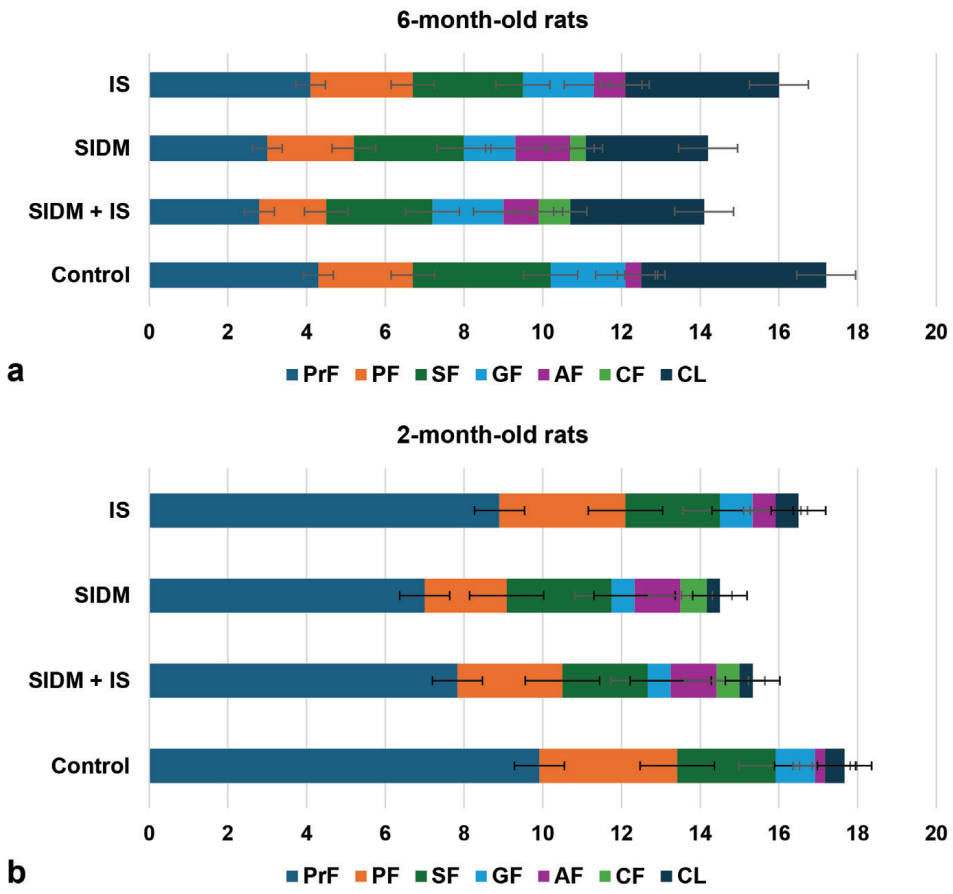

Fig. 2. Quantitative characteristics of the ovaries of mature (a) and peripubertal (b) rats

Notes: PrF – primordial follicles; PF – primary follicles; SF – secondary follicles; GF – Graafian follicles; AF – atretic follicles; CF – cystic follicles; CL – corpus luteum.

reflected in a larger pool of primordial and primary follicles and the near absence of corpus luteum. Modelling of SIDM in this age group of rats, as in sexually mature rats, led to a reduction in the number of primordial and primary follicles compared with control values (Fig. 2). We detected isolated cystic follicles in 3 animals: in 2 with comorbid pathology and in 1 with SIDM. In rats exposed to a single stressor, no significant quantitative changes in the ovarian cortex were observed (Fig. 2). The number of Graafian follicles and corpus luteum in all study groups remained at control levels, indicating that the ovulation process was preserved.

In the control group of 2-month-old rats, ERα expression showed a marked difference compared with 6-month-old animals (Fig. 1a, 3a), namely, isolated interstitial cells exhibited weakly positive expression. In rats with SIDM, weakly positive ERα expression was detected in interstitial cells and theca cells (Fig. 3f, Table 3). In rats of Group 1, ERα expression was more pronounced and was detected in theca cells of primary, secondary and atretic follicles, as well as in Graafian follicles (Fig. 3d). In rats from Group 3, ERα expression could not be detected in ovarian tissue (Fig. 3h, Table 3).

ER mediate the physiological effects of the hormone estrogen on various target organs [23–25]. There are 2 types of ER based on their location: nuclear (nER) and membrane (mER). The nER subtypes are ERα and ERβ [26]. ERα is predominantly localised in the target tissues of estradiol – the uterus, vagina, ovaries, fallopian tubes, pituitary gland and mammary gland – and may also be found in other organs such as the hypothalamus, liver, and components of the cardiovascular system [18, 26, 27]. ERβ, in contrast, is present in a broader range of tissues, including the prostate, salivary glands, testes, ovaries, vascular endothelium, smooth muscle, immune cells, as well as specific neurons within both the central and peripheral nervous systems [26, 28–30]. Both forms of ERα and ERβ are expressed in the mammalian ovary but are localised in different functional compartments. In the human ovary, ERβ expression is particularly pronounced in granulosa cells across all stages of follicular development [31]. ERα is predominantly localised in the interstitium, theca cells and germinal epithelium cells [32]. In the ovary, ERα and ERβ perform distinct but complementary functions in the regulation of folliculogenesis. ERα plays an important role in stimulating granulosa cell proliferation and regulating pituitary gonadotropin secretion, thereby supporting follicular growth and the ovulation process. The activity of this receptor may be enhanced by exogenous gonadotropic stimulation, which is important for the correction of ovulatory disorders.

In contrast, ERβ is regarded as one of the key transcriptional regulators in granulosa cells, as it promotes their differentiation, maintains follicular homeostasis and the normal course of ovulation, and exerts anti-proliferative and anti-inflammatory effects. Furthermore, ERβ is involved in regulating the expression of steroidogenesis genes via sig-

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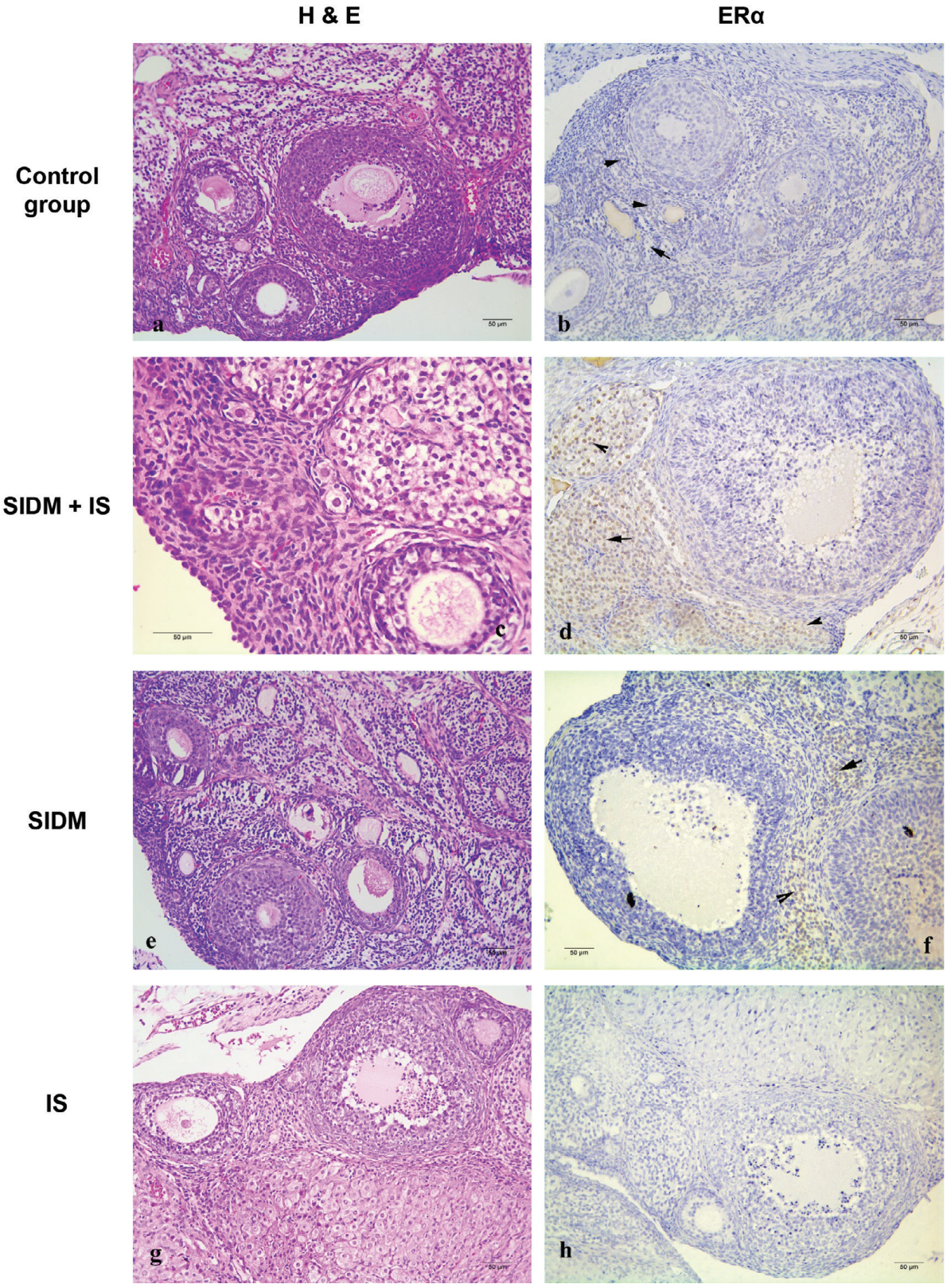

Fig. 3. Ovarian nests and follicles at different stages of maturation in the ovaries of 2-month-old rats (a). Features of ERα expression (arrow) in the ovaries of 2-month-old rats (b). A reduced pool of primordial follicles (c) and aberrant ERα expression (arrow) (d) in Group 1. Impaired folliculogenesis (e) accompanied by decreased ERα (arrow) expression (f) in Group 2 rats. Preservation of ovarian histoarchitecture (g) with a marked reduction in ERα expression (arrow) (h) in Group 3 rats

Notes: H & E – hematoxylin and eosin; ERα – estrogen receptor alpha; SIDM – streptozotocin-induced diabetes mellitus; IS – immobilization stress.

Table 3

Immunohistochemical evaluation of ERα in the ovary of peripubertal rats

Follicular development	Cell type	Research groups			
		Control	SIDM + IS	SIDM	IS
Primordial	Oocyte	–	–	–	–
	Follicular cells	–	–	–	–
Primary	Oocyte	–	–	–	–
	Granulosa cells	–	–	–	–
	Theca cells	–	+	+	+/–
Secondary	Oocyte	–	–	–	–
	Granulosa cells	–	–	–	–
	Theca interna	+	++	+	–
	Theca externa	+	+	–	–
Graafian	Oocyte	–	–	–	–
	Granulosa cells	–	–	–	–
	Theca interna	+	++	+	–
	Theca externa	–	++	–	–
Atretic	Oocyte	–	–	–	–
	Granulosa cells	–	+	+	–
	Fibrous theca	–	++	+	–
Corpus luteum	Luteal cells	–	–	–	–
Interstitium		+	+++	++	–

Notes: ERα expression levels in individual structures are presented as follows: 0 – no staining; 1 (+/–) – very weak staining; 2 (+) – weak staining; 3 (++) – moderate staining; 4 (+++) – intense staining; H & E – hematoxylin and eosin; ERα – estrogen receptor alpha; SIDM – streptozotocin-induced diabetes mellitus; IS – immobilization stress.

naling mechanisms associated with protein phosphorylation, and also influences ovarian sensitivity to gonadotropins, in particular through the modification of the expression of the c-Fos transcription factor [33–35]. Despite the accumulation of scientific data, the precise mechanisms of action of estrogens in the human ovary and their role in the regulation of folliculogenesis remain the subject of further research.

According to our studies in peripubertal rats, ERα expression is significantly lower in ovarian tissues compared with 6-month-old rats. This difference can evidently be explained by the sexual immaturity of the ovaries, since in peripubertal rats the processes of ovulation and corpus luteum maturation are only just beginning to develop; incidentally, the corpus luteum was detected in only 2 of the 5 animals studied. Our findings are also supported by data from other researchers, who report that adult females with ERα knockout (αERKO) are anovulatory, possess pre- and small antral follicles, but lack a corpus luteum, leading to infertility [20, 36, 37].

The findings regarding the increased expression of ERα in 6-month-old rats with SIDM and IS compared to the control group, as well as its abnormal localization (granulosa cells of the cumulus oophorus of Graafian follicles), proved to be of interest. We found no confirmation or refutation of our findings in the literature. However, scientific studies indicate that in PCOS, ER is characterised by ERα dominance and reduced expression of ERβ and GPER1 (G protein-coupled estrogen receptor 1) in the ovaries, hypothalamus and endometrium. The dominance of ERα may underlie key features of the pathophysiology of PCOS, including impaired gonadotropin regulation, anovulation, endometrial hyperplasia and impaired folliculogenesis [33, 38, 39]. Indeed, in this group of rats and in rats with SIDM, the histological picture of the ovaries resembled that seen in PCOS; however, in rats with SIDM, ERα expression was lower than in the first and control groups. However, in rats with SIDM, as in the first experimental group, abnormal localisation of ERα was observed. Some authors point to increased secretion of androstenedione in follicles with ERα deficiency *in vitro* [40]. According to their studies, ERα may reduce androgen production in theca cells by inhibiting CYP17A1. When ER signaling within follicles is disturbed, the responsiveness of theca cells to estrogens may change, which can lead to ovarian hyperandrogenism. Such dysregulation is considered an important factor in the pathogenesis of PCOS [41].

In DM, there is an impairment in the expression of ERs and their signaling pathways. Metabolic disorders, hyperglycemia and oxidative stress can alter the transcription of the *ESR1* (estrogen receptor 1) gene, leading to reduced levels of ERα in tissues. ERα is also involved in the regulation of glucose metabolism and insulin signaling (via the stabilisation of IRS-1 (insulin receptor substrate 1) and the activation of AKT (Protein kinase B)). Disruption of this pathway in diabetes leads to dysfunction of cellular signaling cascades and may alter the response of tissues to estrogens [42]. We also observed a reduction in ERα levels in 6-month-old rats with SIDM in our studies; however, in rats with SIDM exposed to a single dose of IS, increased and abnormal ERα expression was noted in ovarian tissue. It is evident that stress leads to cellular signaling cascades and may alter the response of granulosa and theca cells to estrogens [43]. Moreover, in both prepubertal and sexually mature rats, a single exposure to IS

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led to a significant reduction in ERα expression in ovarian tissue and to abnormal ERα expression in rats with comorbid pathology. Consequently, we observed impaired folliculogenesis with a reduction in the pool of Graafian follicles and corpus luteum, and the appearance of cystic follicles.

A slight increase in ERα expression in 2-month-old rats with DM may lead to ovarian androgenisation. This endocrine imbalance can disrupt follicular development by inhibiting the selection of dominant follicles, ultimately resulting in anovulation and disturbances of the estrous cycle [44]. According to our studies, in Group 2, a decrease in the number of primary and secondary follicles and Graafian follicles was observed, which may indicate delayed sexual maturation.

CONCLUSIONS

In healthy 2-month-old rats, ERα expression was significantly lower compared with that in 6-month-old animals. Weakly ERα-positive cells were detected in the interstitium and theca cells of secondary follicles, whereas in 6-month-old rats, theca cells of all follicles demonstrated intense and moderate positive expression of ERα.

In SIDM, there is a reduction in and abnormal expression of ERα in animals of various ages, accompanied by disturbances in folliculogenesis. In sexually mature individuals, this manifests as a reduction in the pool of primordial follicles, Graafian follicles and corpus luteum, an increase in the number of atretic follicles and the appearance of cystic follicles, indicating depletion of the ovarian reserve and a possible reduction in fertility in this group. In 2-month-old rats, we observed a reduction in the number of secondary follicles and Graafian follicles and the appearance of cystic follicles, which may indicate delayed sexual maturation.

A single exposure to stress leads to a reduction in ERα expression, which is particularly pronounced in peripubertal rats. However, no quantitative changes in ovarian cytoarchitectonics are observed. In contrast, the combination of SIDM and IS leads to increased abnormal ERα expression, accompanied by marked changes in folliculogenesis in both age groups.

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